

FRUIT AND FRUIT PRODUCTS

Detection of High Fructose Corn Syrup in Apple Juice by Mass Spectrometric $^{13}\text{C}/^{12}\text{C}$ Analysis: Collaborative Study

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The addition of high fructose corn syrups (HFCSs) to apple juice can be detected by mass spectrometric determination of $^{13}\text{C}/^{12}\text{C}$ ratios. Forty-one pure apple juice samples, representing 18 varieties, gave a mean value for $\delta^{13}\text{C}$ of -25.3‰ (parts per thousand), while 4 HFCSs averaged -9.7‰ . In a collaborative study, 1 pure apple juice and 4 apple juice-HFCS mixtures containing from 25 to 70% apple juice were properly classified by 6 laboratories. Samples with $\delta^{13}\text{C}$ values less negative than -20.2‰ , 4 standard deviations from the mean of pure juices, can, with a high degree of confidence, be classified as adulterated. Because of the range of values for pure apple juices, other interpretations of $\delta^{13}\text{C}$ data on suspect samples are meaningless (i.e., samples with values more negative than -20.2‰ must be considered unadulterated with HFCS). The $^{13}\text{C}/^{12}\text{C}$ mass spectrometric method for corn syrup products, 31.150-31.153, has been adopted official first action for detecting HFCS in apple juice.

Both economic and seasonal incentives exist for producers of apple juice and apple juice concentrates to adulterate these products and mislabel them as containing no added sugar. Apple juice is now the top selling canned and bottled fruit juice, with 1978 sales of \$224 million, up 30% from the previous year. By adulterating apple juice at the 50% level, the manufacturer can reduce his costs by 40%. Adulteration would be most likely to occur in spring and summer, to stretch the supply of apples, which ripen in the fall. These practices are a threat to the integrity of apple juice markets and represent a fraud to the consumer.

Mixtures simulating natural apple juice are readily prepared by adding corn or cane-derived sugar syrups, artificial and natural flavorings, and citric acid to apple juice, and then diluting with water to the appropriate solids level, about 12%. The sugars fructose, sucrose, and glucose account for over 95% of the solids in apple juice, and the following ranges have been determined for each sugar: fructose, 5.7-7.25%; sucrose, 1.1-3.2%; and glucose, 2.2-3.1% (1, 2). These compositional variations of sugars in apple juice render sugar analyses of little use as an indicator of adulteration, because the low-cost sweetener high fructose corn syrup (HFCS) from a number of manufacturers contains fructose and glucose in different ratios. HFCS would be the likely adulterant, because both its sugar composition and its sweetness would be close to that of apple juice.

Recent evidence that adulteration of apple juice has been occurring on a large scale has been obtained by applying the method of stable carbon isotope ratio analysis, which has been adopted official final action for detecting adulteration of honey with HFCS (3, 4). In one informal study (5), 7 of New England's best-selling apple juices, all labeled as containing no added sugar, were purchased from supermarkets and tested. The results indicated that 4 of the brands contained between 35 and 70% added sugar. In a comprehensive survey (H. W. Krueger & R. H. Reese, in preparation) of several hundred apple juice and apple juice concentrate samples, over one third were found to be adulterated, again by determining $^{13}\text{C}/^{12}\text{C}$ ratios.

The $^{13}\text{C}/^{12}\text{C}$ mass spectrometric method is applicable because the $^{13}\text{C}/^{12}\text{C}$ ratio of a plant-derived material reflects the pathway of photosynthesis in the source plant. Plants using the Calvin (C_3) photosynthetic cycle, including honey floral sources (6), possess $\delta^{13}\text{C}$ values of -22 to -33‰ (parts per thousand); those using

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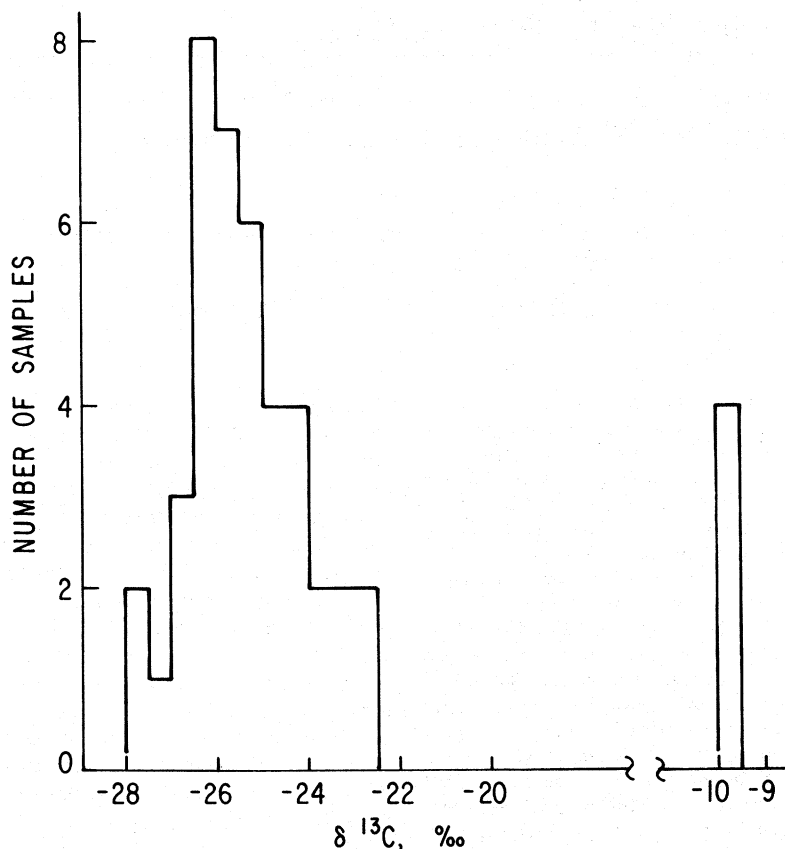


Figure 1. Distribution of $\delta^{13}\text{C}$ values among 41 apple juice samples and 4 HFCS samples.

the Hatch-Slack (C_4) cycle have $\delta^{13}\text{C}$ values ranging from -10 to -20‰ (7, 8). Corn, sorghum, and sugar cane are examples of Hatch-Slack plants, and organic materials derived from them, such as sugars, are enriched in ^{13}C compared with Calvin plants.

A previous report (9) established that $\delta^{13}\text{C}$ values of pure apple juices are quite uniform. Forty samples were analyzed (the 41st was used in the present collaborative study), representing the 18 commercially most significant apple varieties. The values are summarized in Table 1; their distribution is shown in Figure 1. The

mean value of -25.3‰ is very close to that found earlier (3) for 119 honey samples (-25.4‰). No relationship was found between $\delta^{13}\text{C}$ value and variety or geographical origin of the apples, and, like honey, all apples come from C_3 plants. The probability of a given apple juice sample being pure may be determined from multiples of the standard deviation (SD) in Table 2. A sample with a value less negative than -20.2‰ should be classified as adulterated. Because of the range of values found for pure juices (-22.5‰ to -27.9‰), a statistical approach is necessary to interpret the results of this test.

Table 1. $\delta^{13}\text{C}$ Values of apple juices and HFCSs^a

Sample	No. of samples	Mean, ‰	Range, ‰		Std dev., %	Coeff. of var., %
			Low	High		
Apple juice	41	-25.3	-27.9	-22.5	1.275	5.04
HFCS	4	-9.7	-9.8	-9.5	0.14	1.4

^a See Ref. 9 for individual values for 40 apple juice samples, classified by variety. Remaining sample is that used in present collaborative study. Determined by Geochron Laboratories Division, Krueger Enterprises, Inc., Cambridge, MA 02139.

Table 2. Probability of $\delta^{13}\text{C}$ value of authentic apple juice sample being more negative than stated limit

Probability, %	Limit of $\delta^{13}\text{C}$, ‰
5 of 6 = 84.1	-24.1
43 of 44 = 97.72	-22.8
769 of 770 = 99.87	-21.5
24 999 of 25 000 = 99.997	-20.2

The present collaborative study demonstrates that the stable carbon isotope procedure can be used to advantage in detecting the addition of corn sweeteners to apple juice.

Experimental

Materials

Pure apple juice (MacIntosh) was obtained from New England Apple Products Co. (Littleton, MA), and HFCS was obtained from A.E. Staley Mfg. Co. (Decatur, IL). Mixtures for the collaborative study were prepared by diluting the HFCS (73.27% solids) with water to 11.33% solids, the same as that of the apple juice, then combining various proportions, by weight, to give different levels of adulterations. After the samples were autoclaved 30 min, 10 mL of each was added to a screw-top vial, and then sent to collaborators.

Five samples, labeled A through E and containing different proportions of apple juice and HFCS, were sent to 6 laboratories. The content of apple juice in the samples was as follows: A, 55.0%; B, 100.0%; C, 25.0%; D, 40.0%; and E, 70.0%.

Sample Combustion and Determination of ^{13}C

In laboratories with isotope ratio mass spectrometers, various methods are used to combust materials to carbon dioxide before $^{13}\text{C}/^{12}\text{C}$ measurement. Overall accuracy, including combustion and $^{13}\text{C}/^{12}\text{C}$ determination, is 0.3‰ or better. The instruments, combustion conditions, and standards used by the collaborators in the present study are given in Table 3. Working standards also vary among laboratories, but in all cases,

$\delta^{13}\text{C}$ (‰) values are reported from comparisons with carbon dioxide generated from the reference standard PeeDee belemnite (PDB) and calculated from the following formula after comparison of the $^{13}\text{C}^{16}\text{O}_2$ and $^{12}\text{C}^{16}\text{O}_2$ ion beams:

$$\delta^{13}\text{C}(\text{‰}) = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{PDB}}} \right] - 1 \times 10^3$$

A $\delta^{13}\text{C}$ value of -25‰ means that the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample is 2.5% less than that of the PDB standard ($^{13}\text{C}/^{12}\text{C}_{\text{PDB}} = 0.011237$). Corrections are applied to the measured differences, including any zero enrichment in the capillary inlet system, valve mixing between sample and standard valves, and tailing of major onto minor peak signal; 4 collaborators also accounted for the contribution of ^{17}O to mass 45 signal. Collaborators 2 and 3 freeze-dried the samples to thick syrups when they were received; this stabilizes the samples and is recommended. They applied no correction for ^{17}O contribution, because this effect, due to large amount of water in apple juice samples, was greatly minimized.

Corn Syrup in Apple Juice

Carbon Ratio Mass Spectrometric Method Official First Action

Principle

Sample is burned completely to CO_2 and H_2O ; CO_2 is purified, and ^{13}C to ^{12}C ratio is measured in isotope ratio mass spectrometer. Difference in $^{13}\text{C}/^{12}\text{C}$ values for apple juice (av. $\delta^{13}\text{C} = -25.3\text{‰}$) (parts per thousand) and corn syrup (av. $\delta^{13}\text{C} = -9.7\text{‰}$) provides measure of corn syrup (including high fructose corn syrup (HFCS)) in apple juice.

Apparatus

(a) *Combustion system*.—Vac.-tight glass manifold including quartz combustion tube $\frac{1}{2}$ filled with CuO in tubular furnace, liq. N trap, automatic Toepler pump, and high-vac. source.

(b) *Purification system*.—Glass manifold interconnected with combustion system including trap, sample

Table 3. Instruments and conditions used in collaborative study

Coll.	Mass spectrometer	Combustion		Working std ^a
		Temp., °C	Recirculation time, min	
1	Micromass 602D	850	10	Charcoal ($\delta^{13}\text{C} = -23.3\text{‰}$ vs PDB) and marble ($\delta^{13}\text{C} = 0.0\text{‰}$ vs PDB) calibrated against NBS-20 Solenhofen limestone ($\delta^{13}\text{C} = -1.06\text{‰}$ vs PDB), and NBS-22 oil ($\delta^{13}\text{C} = -29.5\text{‰}$ vs PDB)
2	Micromass 602D	850	10	AER vacuum pump oil ($\delta^{13}\text{C} = -27.32\text{‰}$ vs PDB)
3	Micromass 602C	1200	—	UQ2 marble ($\delta^{13}\text{C} = +1.91\text{‰}$ vs PDB)
4	Nier type-6 in. 60° sector-dual collecting	800	20	NBS-22 oil ($\delta^{13}\text{C} = -29.5\text{‰}$ vs PDB) and NBS-21 graphite ($\delta^{13}\text{C} = -27.8\text{‰}$ vs PDB)
5	Micromass 602	> 1500	—	Oxalic acid and Bender limestone
6	MAT 250, Varian	800	—	Flask- CO_2 vs PDB

^a NBS = National Bureau of Standards reference material.

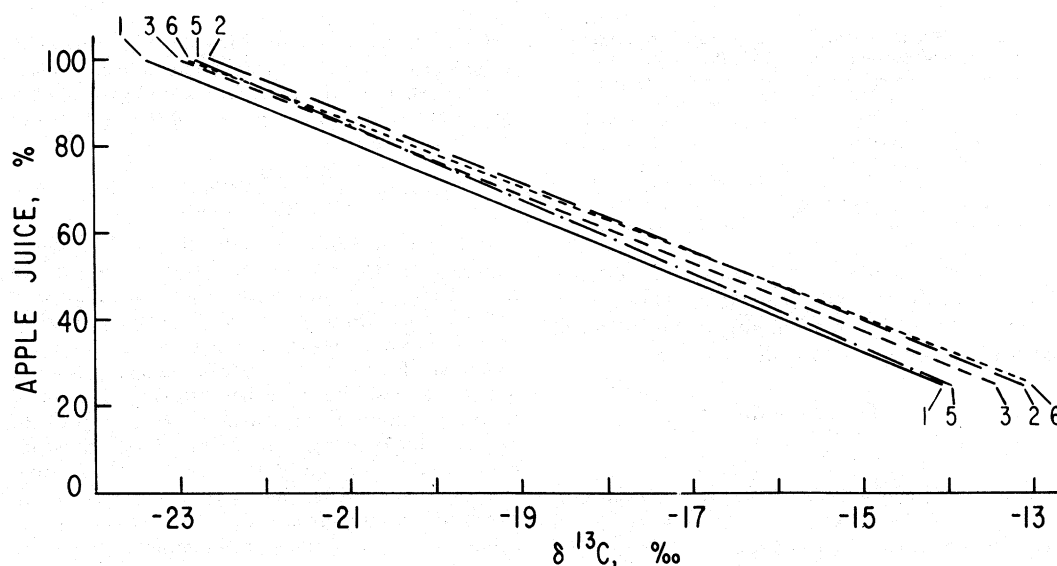


Figure 2. Graphic presentation of collaborative study results (excluding Collaborator 4).

collection tube, and manometer (see Fig. 31:02 and *Geochimica et Cosmochimica Acta* 3, 54-55(1953)).

(c) *Mass spectrometer*.—Micromass 602 (new model 602D) (Kearns Groups, 58 Buckingham Dr, Stamford, CT 06902), Nuclide 6-60-RMS (Nuclide, 642 E College Ave, State College, PA 16801), Varian MAT G D150 (superceded by MAT 250) (Varian MAT Mass Spectrometry, 25 Hanover Rd., Florham Park, NJ 07932), or equiv. instrument designed or modified for isotope

ratio measurement and capable of accuracy of 0.01% of abundance at mass 45.

Preparation of Sample

Apple juice samples may be freeze-dried to thick syrup for stabilization.

Place 300-400 mg sample, weighed to nearest 0.1 mg, in ceramic boat, position boat in tube, and evacuate system. Admit to 600 mm Hg, tank O purified over

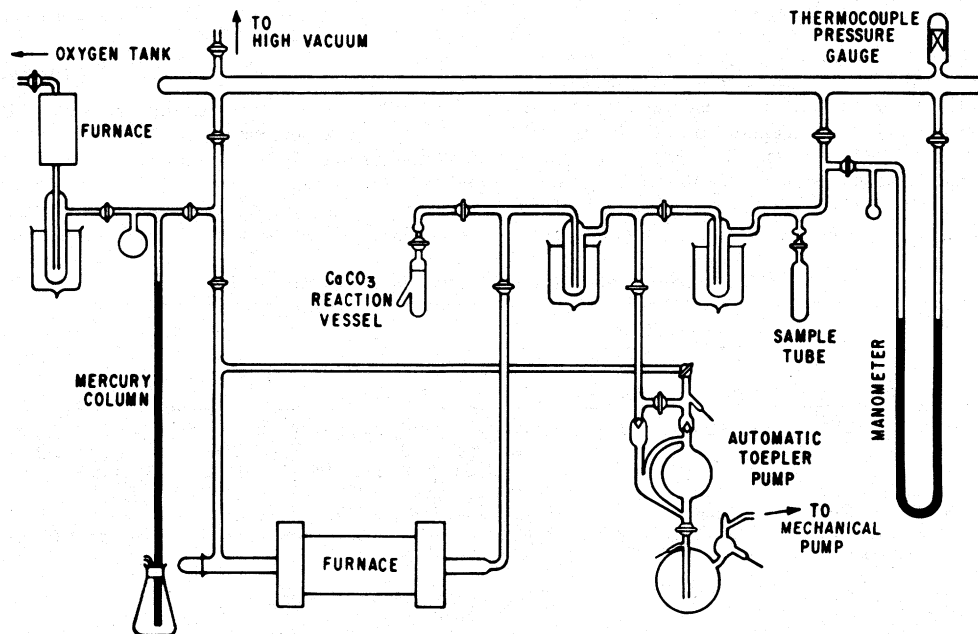


Figure 31:02. Carbon combustion and purification system.

CuO at 700°, followed by liq. N trap. Heat sample to ≥850° in manifold in tubular furnace, condensing CO₂ in liq. N trap. Recirculate gases over CuO 10–30 min at 850°. Isolate collection trap and purification system from combustion system and Toepler pump by valves, and pump off O. Cool purification trap with solid CO₂-acetone; cool sample tube with liq. N. Let collection trap warm, condensing impurities in solid CO₂ trap and CO₂ in sample tube.

Determination

Operate mass spectrometer according to manufacturer's instructions. Calibrate with ≥2 stds such as NBS SRM 20 Solenhofen limestone ($\delta^{13}\text{C} = -1.06\text{‰}$ against Pee Dee belemnite (PDB)), NBS SRM 21 graphite ($\delta^{13}\text{C} = -27.8\text{‰}$), or NBS SRM 22 crude oil ($\delta^{13}\text{C} = -29.5\text{‰}$). Correct values obtained for zero enrichment in inlet system, mixing between sampling and std valves, tailing of major onto minor peak signal, and contribution of ¹⁷O to mass 45 signal. Calc.:

$$\delta^{13}\text{C}(\text{‰}) = \left[\frac{^{13}\text{C}/^{12}\text{C sample}}{^{13}\text{C}/^{12}\text{C std}} \right] - 1 \times 1000.$$

Convert laboratory analyses, relative to whatever std was used, to PDB base by following relationship:

$$\delta_{(X-PDB)} = \delta_{(X-B)} + \delta_{(B-PDB)} + 10^{-3} \delta_{(X-B)} \times \delta_{(B-PDB)}$$

where (X – B) and (X – PDB) refer to analyses of sample (X) relative to std (B) and relative to PDB, and (B – PDB) is analysis of std (B) relative to PDB, all δ 's in parts per thousand.

Sample with $\delta^{13}\text{C}$ value less negative than –20.2‰ relative to PDB is considered adulterated.

Results and Recommendations

The results of the collaborative study are compiled in Table 4. The results of Collaborator 4 for Samples B and C were quite different from those of the other collaborators, but all adulterated samples in the study (A, C, D, E) would be properly classified, according to the highest probability limit set in Table 2. With use of the other limits, Sample B (pure apple juice) of Collaborator 4 would be classified as adulterated. Figure 2 shows regression lines of % apple juice on $\delta^{13}\text{C}$ for all collaborators except for Collabo-

Table 4. $\delta^{13}\text{C}$ (‰ vs PDB) for collaborative samples

Coll.	Sample				
	A	B	C	D	E
1	–17.8	–23.4	–14.0	–16.1	–19.7
2	–17.0	–22.9	–13.2	–15.1	–18.9
3	–17.4	–23.0	–13.2	–15.4	–19.2
4	–17.5	–20.8	–16.9	–16.0	–19.5
5	–17.7	–22.5	–13.8	–15.5	–19.4
6	–16.9	–22.9	–13.1	–15.0	–19.0
Mean	–17.4	–22.6	–14.0	–15.5	–19.3

Table 5. Statistical evaluation of data in Table 4

Statistic	Pairs	
	C-D	A-E
All Collaborators		
S_d	1.27	0.466
S_r	0.82	0.10
S_b	0.69	0.32
$F^a(5 \text{ DF})$	2.40	15.90**
Excluding Collaborator 4		
S_d	0.58	0.51
S_r	0.14	0.11
S_b	0.40	0.35
$F^a(5 \text{ DF})$	17.68**	23.18**

^a For presence of systematic errors. DF = degrees of freedom.

** Significant with $P < 0.01$.

rator 4. These lines were all significant ($P < 0.01$) with very high correlation coefficients, $-1.00 < r < -0.99$.

The procedures described by Youden (10) were applied to the results in Table 4. For this analysis, Sample A was paired with E, and C with D. The results are given in Table 5, with and without the data of Collaborator 4. Exclusion of Collaborator 4 only serves to reduce the estimate of S_r in pair C-D. It does not eliminate the presence of systematic error that the F -test indicates is present.

Independent estimates of repeatability (S_r) give a value of 0.31. This is obtained from the results of Collaborators 2, 3, and 5, who provided duplicate measurements.

From these results, the Associate Referee recommends that the ¹³C/¹²C mass spectrometric method be adopted official first action for detecting HFCS in apple juice.

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